



NTP
National Toxicology Program

**Toxicology and Carcinogenesis Studies of
Milk Thistle Extract in F344/N Rats and B6C3F1 Mice
(Feed Studies)**

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NTP Board of Scientific Counselors
Technical Reports Review Subcommittee
November 19, 2009





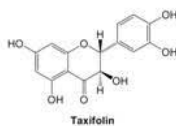
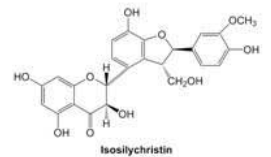
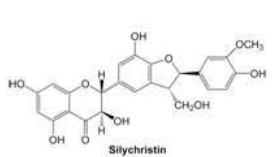
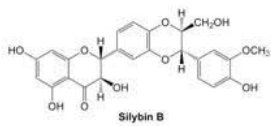
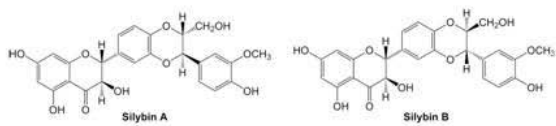
Nomination of Milk Thistle

- Nominated by NIEHS as part of the herbal medicine initiative
 - Used in folk medicine to treat liver disease
 - ~ 3.4% of adults in the U. S. use milk thistle
 - Lack of toxicity or carcinogenicity information
- Ongoing NIH Clinical Trials with milk thistle
<http://clinicaltrials.gov/ct2/results?term=milk+thistle>





Major Components of Milk Thistle





Alkaloid Composition of Milk Thistle Extract

	Approximate Retention Time	Lot: 27691/M6 2-year	Lot: 27007/M1 13-week
Component	minutes	% Total Area ¹	% Total Area ²
Taxafofin	8.5	5.0	3.6
Isosilychristin	11.8	3.0	2.9
Unidentified	12.7	2.2	1.7
Silychristin	13.2	18.7	21.2
Silydianin	14.3	10.8	2.7
Silybin A	19.2	17.1	22.8
Silybin B	19.9	28.1	34.6
Isosilybin A	21.6	8.8	7.4
Isosilybin B	22.0	3.6	2.6
Unidentified	22.2	0.5	0.5

¹ 10 additional unidentified peaks with peak areas less than 0.5%, totaling 2.1% of the total area were found in Lot M27691/M6

² 8 additional unidentified peaks with peak areas less than 0.5%, totaling 1.7% of the total area were found in Lot M27007/M1



Genotoxicity Test Results

- Salmonella studies (Lot: 27691/M6)
 - Negative in strains TA97, TA100, TA102, TA104, TA1535 with/without activation
 - Positive in TA98 with S9 activation; negative without S9
- Micronucleus assay, male and female mice (3-month study)
 - Negative



Milk Thistle Extract Experimental Study Design

Study Animals: Male and Female F344/N Rats & B6C3F1 Mice

Route: Feed

13-Week studies: 0, 3125, 6250, 12,500, 25,000, 50,000 ppm
10 animals/species/sex/dose

2-Year studies: 0, 12,500, 25,000, 50,000 ppm
50 animals/species/sex/dose



13-Week Studies - Survival and Body Weights Rats and Mice

- All rats & mice survived
- Final mean body weights of treated groups of rats & mice within $\pm 10\%$ of controls



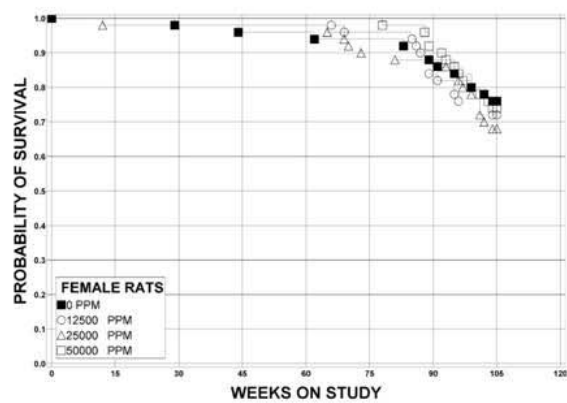
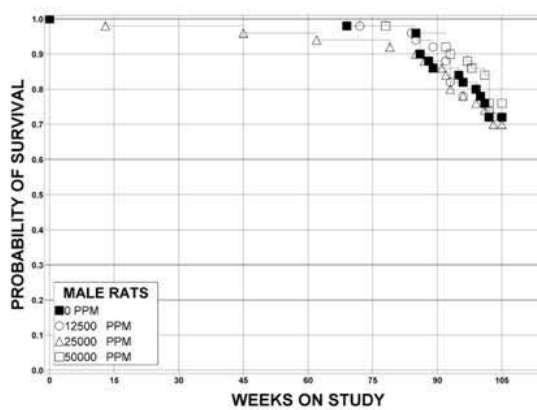
13-Week Studies - Treatment - Related Lesions Rats and Mice

Dose (ppm)	0	3125	6250	12,500	25,000	50,000
Male Mice						
Forestomach:						
hyperplasia	0	0	0	0	2	1
ulcer	0	0	0	0	1	1
Female Mice						
Forestomach:						
hyperplasia	0	0	0	0	2	2
ulcer	0	0	0	0	0	1

N = 10 per group

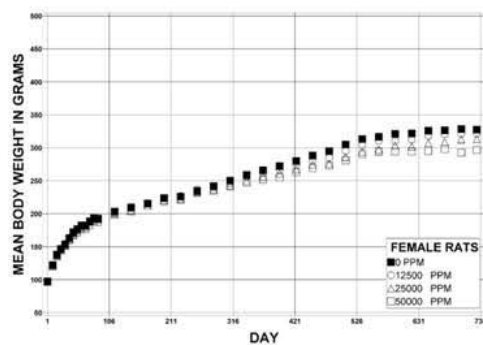
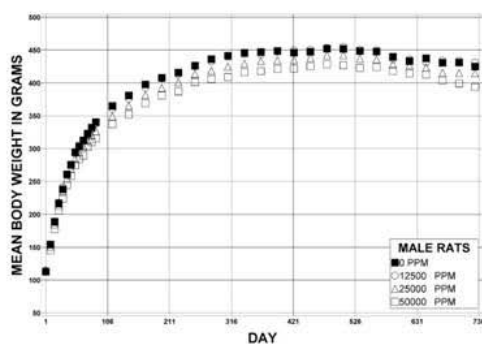


Survival - 2-year Rat Study





Body Weights - 2-year Rat Study





Selected Liver Lesions - Rats

Dose (ppm)	0	12,500	25,000	50,000
Male Rat				
Bile duct, hyperplasia	50 (2.5) ^a	48 (2.4)	44 (2.1)	17** (1.4)
Infiltration cellular, mixed cell	31 (1.1)	32 (1.0)	27 (1.1)	15** (1.0)
Female Rat				
Bile duct, hyperplasia	37 (1.4)	10** (1.7)	10** (1.3)	8** (1.1)
Clear cell focus	9	10	17*	21*
Mixed cell focus	3	6	11*	10*

* p< 0.05; ** p<0.01

^aSeverity of lesion

N = 50 per group



Mammary Gland Lesions - Female Rat

Dose (ppm)	0	12,500	25,000	50,000
Fibroadenoma	28**	27	17	18*
Mammary gland fibroma, fibroadenoma, adenoma, carcinoma ^a	28* (56%)	27 (54%)	19* (38%)	20* (40%)

*p<0.05; ** p<0.01

p value for trend statistic under control column

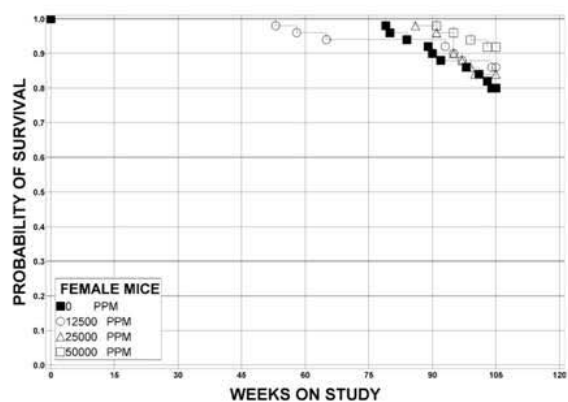
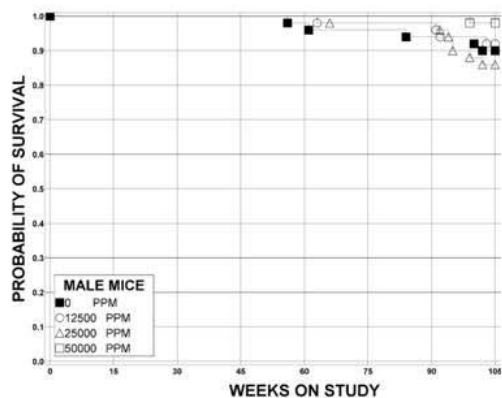
^aHistorical control rate, feed

57% ± 5.3% (50%-62%)

N = 50 per group

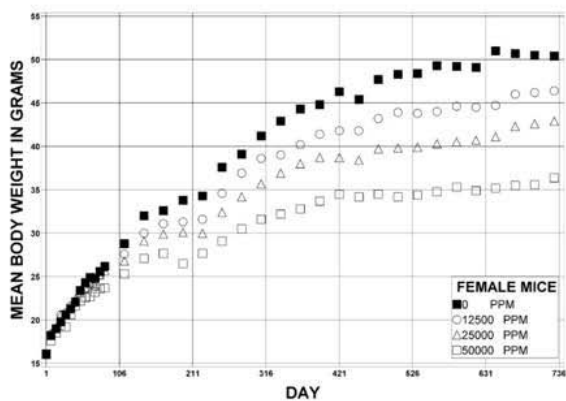
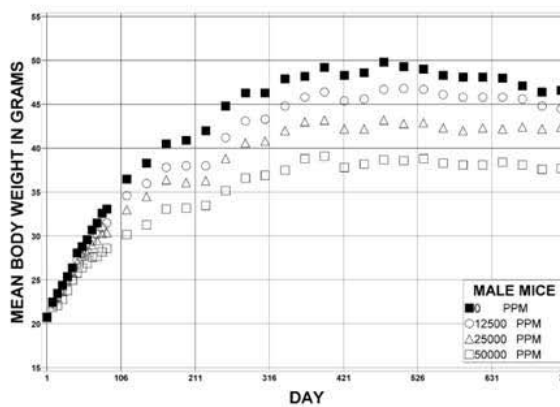


Survival - 2-year Mouse Study





Body Weights - 2-year Mouse Study





Selected Liver Lesions - Male Mouse

Dose (ppm)	0	12,500	25,000	50,000
Hepatocellular adenoma	12**	13	5	1**
Hepatocellular carcinoma	17**	15	14	7*
Hepatocellular adenoma or carcinoma ^a	26** (52%)	22 (44%)	16 (32%)	8** (16%)

*p<0.05; ** p<0.01

p value for trend statistic under control column

^aHistorical control rate, feed

53.5% ± 7.6% (46%-64%)

N = 50 per group



Selected Liver and Spleen Lesions - Male Mouse

Dose (ppm)	0	12,500	25,000	50,000
Hepatocyte vacuolization cytoplasmic	7	4	1	0**
Hepatic Clear cell foci	7	3	3	0**
Hepatic Mixed cell foci	10	7	0	3*
Spleen, hematopoietic cell proliferation	16	11	12	4**

*p<0.05; ** p<0.01

N = 50 per group



Conclusions

- Male Rats: **No** evidence of carcinogenic activity
- Female Rats: **No** evidence of carcinogenic activity
- Male Mice: **No** evidence of carcinogenic activity
- Female Mice: **No** evidence of carcinogenic activity

- Administration of Milk Thistle extract for 2-years resulted in:
 - Liver: increases in clear cell and mixed cell foci in Female Rats
 - Decreased body weights in Male and Female Mice
 - Decreases in mammary gland neoplasms in Female Mice
 - Decreases in hepatocellular neoplasms in Male Mice